Microbial Characterization of Free Floating Condensate aboard the Mir Space Station

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Abstract

Three samples of humidity condensate that had accumulated behind panels aboard the Russian space station Mir were collected and returned to earth for analysis. As these floating masses of liquid come into contact with the astronauts and the engineering systems, they have the potential to affect both crew health and systems performance. Using a combination of culturing techniques, a wide variety of organisms were isolated included *Escherichia coli*, *Serratia marcescens*, and a presumed *Legionella* species. In addition, microscopic analysis indicated the presence of protozoa, dust mites, and spirochetes. These findings suggest the need for more comprehensive microbial analysis of the environment through the use of new methodologies to allow a more thorough risk assessment of spacecraft.

Introduction

Understanding microbial diversity in complex ecosystems is challenging as few models can control external interference. The study of semiclosed systems, which have limited external influence, can provide insight into basic microbial and human interactions from which a baseline of microbial diversity can be determined. In the late 1990s, a microbial evaluation aboard the Russian space station Mir provided an opportunity for the National Aeronautics and Space Administration (NASA) to evaluate such a semiclosed environment and gain insight into the microbial diversity aboard spacecraft occupied for long periods of time in an effort to mitigate the risk to crew health and ensure systems performance.

Mir, which launched in 1986, incorporated a fiveport docking hub that allowed connection of the Soyuz-

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TM human transport vehicle, the Progress unmanned resupply ship, and additional habitation modules. The initial Mir study focused on environmental samples from the Core Module, containing the control center, dining area, food preparation, sleeping quarters, hygiene facilities, and exercise equipment, and the Kristall Module, containing scientific equipment, retractable solar arrays, and a docking node. Although this planned study provided data on the common biota aboard Mir, a unique opportunity arose first during the NASA 6 mission when a large free-floating mass of water was discovered behind a service panel in the Kvant-2 Module. This "free condensate" accumulated over time as water droplets coalesced in microgravity. Originally not a component of the study, the Kvant-2 Module was a full-size module that provided an area to conduct biological and earth observation research. The Kvant-2 also included a contingent life support system, drinking water, oxygen provisions, and shower and washing facilities. Because of the limited amount of floating condensate available and the logistics of return, only two subsequent samples from the Kvant-2 Module were collected during the subsequent and final NASA mission. All samples were thoroughly analyzed for microorganisms to determine the microbiota that accumulated and survived over time.

Methods

Sample Locations and Collection. The condensate sample locations included sample 1, collected during the NASA 6 mission on January 15, 1998, behind a panel in the Kvant-2 Module; sample 2, collected during the NASA 7 mission on May 28, 1998, from the Kvant-2 Module; and sample 3, collected during the NASA 7 mission on May 28, 1998, from the Kvant-2 Module heat exchanger. Samples were collected using a 60-mL syringe to transfer the liquid into a 300-mL Teflon bag. A cotton-tipped swab was also used for sample collection and

Table 1. Bacteria and fungi isolated from free condensate during NASA Mir 6 and 7

Sample 1	Sample 2	Sample 3
Bacteria		
Alcaligenes eutrophus	Alcaligenes faecalis	Bacillus coagulans
Alcaligenes latus	Bacillus species	Bacillus licheniformis
Citrobacter freundii	Bacillus circulan	Bacillus pumilus
Corynebacterium aquaticum	Bacillus coagulans	Bacillus species
Corynebacterium jeikeium	Bacillus licheniformis	Comamonas acidovorans
Enterobacter agglomerans	Bacillus pumulis	Corynebacterium species
Escherichia coli	Citrobacter brackii	Enterobacter cloacae
Hydrogenophaga flava	Citrobacter freundii	Presumptive Legionella species
Kingella denitrifican	Comamonas acidovorans	Pseudomonas species
Methylobacterium species	Corynebacterium species	Rhodococcus species
Pseudomonas vesicularis	Flavobacterium meningosepticum	Serratia liquefaciens
Serratia liquefaciens	Presumptive Legionella species	Serratia marcerans
Stentrophomonas maltophilia	Pseudomonas fluorescens	Sphingobacterium thalpophilun
	Ralstonia paucula	Yersinia frederiksenii
	Serratia liquefaciens	Yersinia intermedia
	Serratia marcesens	
	Yersinia frederiksenii	
	Yersinia intermedia	
Fungi		
Acremonium species	Candida guilliermondii	Candida guilliermondii
Candida guilliermondii	Candida lipolytica	Candida lipolytica
Candida krusei	Cladosporium species	Fusarium species
Cladosporium species	Fusarium species	Hansenula anomala
Fusarium species	Hansenula anomala	Penicillium species
Penicillium species	Penicillium species	Rhodotorula glutinis
Rhodotorula rubra	Rhodotorula glutinis	Rhodotorula rubra
	Rhodotorula rubra	

stored in phosphate buffer (pH 7.0) for return transfer. Samples were maintained at ambient conditions (≈28°C) until being returned to ground.

Sample Processing. Upon return, a wet mount of the samples was visually examined using light microscopy. Samples were examined for the presence of ova and parasites by use of Kinyoun's Acid-Fast and trichrome stains. Cultures were prepared to determine the presence of aerobic bacteria using blood agar, trypticase soy agar, chocolate agar, R2A, MacConkey agar, phenylethanol agar, buffered charcoal yeast extract with polymixin B (PAC), and buffered charcoal yeast extract with dyes, glycine, polymixin B, and vancomycin (DGVP) (Becton, Dickinson and Company, Franklin Lakes, NJ). Anaerobic cultures were prepared both on blood agar and in thioglycolate broth. Mitchison 7H11 agar, and Middlebrook 7H10 agar were used to evaluate the presence of Mycobacterium. Bacterial cultures were incubated at 35°C and examined after 48 h. (Mycobacterium cultures were held 2-8 weeks.) Fungi were cultured on Sabouraud agar and Sabouraud agar with chloramphenicol, incubated at 25°C, and examined after 5 days. All bacterial isolates were identified with either a Biolog Automated Identification System (Biolog, Hayward, CA) or a VITEK Identification System (bioMérieux, Hazelwood, MO). Fungi were identified microscopically by their morphological

characteristics. An aliquot of 1 mL from each sample was prepared for electron microscopic evaluation as previously described [2].

Results

Samples 1 and 2 were cloudy with a brown particulate matter. Sample 3 was also cloudy, but contained a white particulate matter. The 13 bacterial isolates that were isolated from sample 1 were predominantly Gram negative and included several members of the family Enterobacteriaceae, such as Escherichia coli and Enterobacter agglomerans (Table 1). From sample 2, 18 bacterial species were isolated. As with sample 1, several species from the family Enterobacteriaceae were identified, including two Serratia and two Yersinia species. While sample 1 had no Bacillus species, sample 2 differed, as five species of Bacillus were isolated. From sample 3, 15 bacterial species were isolated. As with sample 2, several species of Bacillus were identified. Bacteria were isolated from samples 2 and 3 on buffered charcoal yeast extract with polymixin B, and buffered charcoal yeast extract with dyes, glycine, polymixin B, and vancomycin. Although this isolation suggested the presence of Legionella species, precise identification was not possible, as the colonies could not be subcultured. The only bacterium common to all three samples was Serratia liquefaciens.

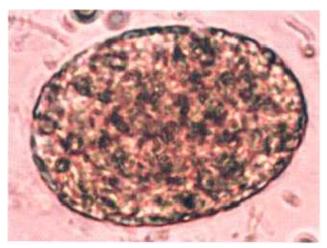


Figure 1. Amoeba recovered from free condensate during NASA 6.

At least seven distinct fungal isolates were identified in each sample. Fungal isolates from all samples included both yeast and filamentous fungi. The organisms Candida guilliermondii and Rhodotorula rubra, as well as a Fusarium species and a Penicillium species, were identified from all samples. No ova, parasites, anaerobic bacteria, or Mycobacterium were detected in any samples.

Microscopic examination revealed the presence of amoeba resembling Acanthamoeba or Hartmanella species (Fig. 1) and ciliated protozoa resembling Stylonychia species in sample 1. Samples 2 and 3 indicated the presence of dust mites (Fig. 2) and ciliated protozoa (Fig. 3). In sample 1, scanning electron micros-

copy suggested the presence of spirochete bacteria and several bacterial rods interconnected in a biofilm glycocalyx.

Discussion

Historically, analyses of spacecraft environments associated with short-duration missions have indicated only a relatively limited microbial diversity with few medically significant organisms present. Preflight environmental testing of air and surfaces from the Space Shuttle program have indicated Staphylococcus, Bacillus, Corynebacterium, and Micrococcus as the predominant bacterial species [3]. Fungal identification from these preflight samples indicated the presence of only filamentous fungi, such as Penicillium, Cladosporium, and Aspergillus species [3]. Environmental data from Mir provided similar information about spacecraft as Staphylococcus, Bacillus and Corynebacterium, and Micrococcus were the most common bacterial isolates from surfaces and air, while Penicillium, Cladosporium, and Aspergillus were the most common fungi isolated [3]. Candida species, primarily C. guillermondii and C. famata, were also commonly isolated from Mir surfaces, whereas yeast were not commonly isolated from the Space Shuttle [3, unpublished NASA reports]. The most common medically significant organism was Aspergillus flavus, which occurred in \sim 50% of the air samples [3]. On average, surface samples displayed fewer than two fungal and two bacterial strains at any given location during a sampling session (unpublished NASA reports). Air samples displayed a slightly higher diversity,

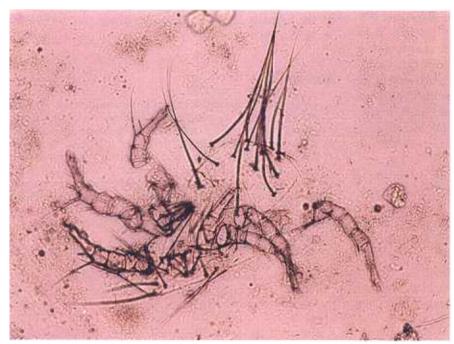


Figure 2. Dust mite recovered from free condensate during NASA 7.



Figure 3. Ciliated protozoa recovered from free condensate during NASA 7.

averaging about four different fungal strains and two different bacterial strains at any given site during a sampling session.

In contrast to the Mir air and surface samples, the free condensate provided a unique opportunity to collect organisms in a liquid medium over a long period of time. While the consortium within the condensate may have selected the organisms that were eventually isolated, their presence in these samples indicated their existence in the air or on surfaces of Mir. The isolation of bacteria from the family Enterobacteriaceae was notable, as isolation of these bacteria either preflight or during flight is rare (unpublished NASA reports). Although E. coli, S. marcesens, or Yersinia intermedia can be medically significant, perhaps the greater question is the source of these organisms. During its tenure in flight, the Mir space station has been exposed to numerous flight experiments with animals and plants, an assortment of payloads, numerous crewmembers, and multiple maintenance activities performed on life support and other systems. The source of these and other contaminants was not determined, but the presence of such organisms reinforces the need for microbial surveillance in closed environments regardless of the disinfection protocols.

Several opportunistic pathogens, such as Stenotrophomonas maltophilia, Ralstonia paucula, C. guilliermondii, and C. krusei, were isolated from the free condensate samples. Some research suggests that spaceflight negatively affects the immune system in both humans and animals [1, 4, 5], which could result in an increased risk of infectious disease events occurring during spaceflight. However, because of the limited incidence of infectious disease, opportunistic pathogens have not been considered a clear medical risk, and remediation based solely upon their presence has never been implemented.

Perhaps the most notable findings were the discovery of protozoa, dust mites, and possibly spirochetes in the free condensate. Also notable was that the number of bacterial and fungal species from each of the free condensate samples was greater than that found by air and surface sampling during flight. Although neither of these observations was remarkable, these and other analytical results of the free condensate indicated a need for a more comprehensive analysis than could be provided by the traditional culture-based techniques that have been the standard for flight missions for the past 30 years. The findings from the free condensate suggested that enhanced methodology, and corresponding disinfection, should be implemented not only for ISS but also for the planetary protection effort as missions with and without crew travel to distant planets. Overall, as the duration of all missions increase, the need for enhanced analytical capabilities will become greater to mitigate the risks to crew health and operational performance of the engineering systems.

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